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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
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SCOTT CROWE et al.)
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Serial No. 07/952,640) Examiner: C. Eisenschenk
)
Filed: December 1, 1992) Group Art Unit: 1806
)
For: PRODUCTION OF ANTIBODIES)

#24 3199S 11-30-94

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Alan Lewis, declare that:

1. I am the same Alan Lewis named as an inventor on the above-referenced patent application.

2. I received my B.Sc. Honors Degree in Biological Sciences in 1984 and my Ph.D in 1987 from the University of Leicester. From 1987-1990 I conducted postdoctoral research in molecular biology at Wellcome Biotech. Since 1990, I have been employed as a molecular biologist in the department of cell biology at the Wellcome Research Laboratories. In this position, my work has focused on carrying out fundamental research in immunochemistry using recombinant DNA technology for the purpose of formulating new therapeutic antibody molecules. A copy of my curriculum vitae is attached.

3. I have read and understand the Office Action issued by the U.S. Patent and Trademark Office on May 27, 1994, in connection with this patent application. In paragraph 18 of the Action, the examiner asserted that claims 1-14, all of the claims pending in the application, stand rejected under 35 U.S.C. § 112, first paragraph, on the basis that the disclosure is not enabling for the use of the claimed invention as a diagnostic aid. More specifically, the examiner stated that the specification does not teach how to use the antibodies made in accordance with the claimed invention as diagnostic aids.

4. This application discloses and claims a process for the production of a recombinant primate antibody. As used in this application, "primate" encompasses humans, apes, old world monkeys, new world monkeys and prosimians. The process of the invention comprises selecting a cell line derived from a primate lymphocyte that is capable of expressing a desired antibody, isolating RNA from that cell line and separating mRNA from the other isolated RNA, synthesizing cDNA from the mRNA and inserting that cDNA into a cloning vector, transforming a host cell with the vector containing the cDNA in order to obtain a library, screening the library for cDNA which encodes the constant and variable regions of the heavy and light chains of the desired antibody, inserting that cDNA encoding the heavy and light chains into an expression vector, and then culturing the transfected host cell under antibody-producing conditions and then isolating the desired antibody.

5. Persons of ordinary skill in the art of immunology will be able to use the recombinant primate antibodies of this invention in various diagnostic assays absent specific instructions for doing so. Specifically, one of skill in the art could replace in a radioimmunoassay (RIA) or an enzyme linked immunosorbent assay (ELISA) the currently used antibody which specifically recognizes a particular antigen with a recombinant primate antibody made in accordance with the teachings of this invention which specifically recognizes that antigen. As these immunoassay techniques are based upon the specific interaction of the antibody with the antigen to provide information about antigenic specificity, the recombinant antibody can be used interchangeably with the hybridoma-produced antibody. Producing the antibody recombinantly as taught in this application does not alter the antibody's ability to bind to the antigen.

6. For example, two diagnostic assays presently on the market include the Wellcozyme™ anti-HAV (hepatitis A virus) immunoassay (VK34) and the HIV (Immunodeficiency Virus) immunoassay (VK56/57), both of which are presently marketed by Murex Diagnostics. Both assays are ELISA assays which utilize a specific anti-viral antibody conjugated to the enzyme horseradish peroxidase to measure the quantity of either anti-HAV antibody or anti-HIV antibody present in serum samples using a standard competitive binding assay. A recombinant primate antibody prepared by the process taught in the present application from a lymphocyte cell line expressing a functional antibody with a

desired specificity for HAV or for HIV easily could be substituted for the HAV or HIV antibodies presently used in these commercial assays. Such a substitution would require no more than routine experimentation to establish that the assay utilizing the recombinant primate antibodies of the present invention functioned reproducibly in the assay.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Alan Lewis

14th November 1994

Date

PA1260

CURRICULUM VITAE

Name: Alan Peter LEWIS

Address: 20 Morgan Road
Bromley
Kent
BR1 3QF

Telephone no: 081 466 6914

Date of Birth: 11th February 1963

Nationality: British

Marital Status: Married

First Degree:

1981 - 1984 University of Leicester

B.Sc. Honours Degree in Biological Sciences, Grade IIi

2nd Year Course Units: Genetics Systems; Molecular Genetics; Genes and Development I; Basic Microbiology; Biology of Non-Chordates; History of Biology.

3rd Year Course Units: Current Topics in Molecular Genetics; Genes and Development II; Animal Cytology; Biochemistry of Gene Expression.

3rd Year Project: "The use of spot hybridisation techniques to search for an *Escherichia coli gyrB*-like DNA sequence in the genomes of various eukaryotic and prokaryotic organisms".

Postgraduate qualifications:

1984 - 1987 University of Leicester, Faculty of Medicine

Ph.D.: "The characterisation of P element-induced singed mutations in *Drosophila melanogaster*, and an analysis of the extent of mobilisation of transposable elements in a P-M hybrid dysgenic cross".

Postdoctoral research:

1987 - 1990

Postdoctoral molecular biologist, Grade 8
Department of Molecular Biology
Wellcome Biotech
Langley Court
Beckenham
Kent, BR3 3BS

Project funded by the World Health Organisation:
"An animal model (*Plasmodium yoelii*) for the development of a vaccine against the asexual blood stage of *Plasmodium falciparum* based on the precursor to the major merozoite surface antigens".

1990 -

Molecular Biologist (presently Grade 10)
Department of Cell Biology
Wellcome Research Laboratories
Langley Court
Beckenham
Kent, BR3 3BS

The major objective of the work is to perform fundamental research in immunochemistry using recombinant DNA technology for the purpose of formulating new therapeutic antibody molecules. In particular, this involves the creation of novel humanised monoclonal antibodies and the rescue of human monoclonal antibodies.

Publication List: See separate sheet.

Interests, hobbies and recreations:

I play the alto saxophone, clarinet, flute, piano and keyboards, having performed in jazz-rock and session groups, and in a number of big bands, including "The Welsh Jazz Orchestra". I enjoy writing and arranging classical, jazz and rock music.

I take an active part in most sports and play badminton and football for the Wellcome (Beckenham) Sports and Social Club.

Full driving licence obtained in February, 1981.

I am a member of British Mensa.

Research Studies:

Postgraduate research

Ph.D.: "The characterisation of P element-induced singed mutations in *Drosophila melanogaster,* and an analysis of the extent of mobilisation of transposable elements in a P-M hybrid dysgenic cross".

The *Drosophila melanogaster* transposable element, P, is linked to the process of P-M hybrid dysgenesis, a syndrome of abnormalities observed in the offspring of a cross between a male of a P factor-containing strain and a female of a strain lacking the elements. The abnormalities are the result of elevated P transposition rates in such a cross.

There has been speculation as to whether other transposable element families are also mobilised during P-M hybrid dysgenesis. Isofemal lines derived from such a cross were analysed by *in situ* hybridisation using cloned copies of the transposable elements *copia, 412* and F. It was found that lines derived from dysgenic crosses showed a statistically significant number of new sites for these elements when compared to a non-dysgenic control. This result suggests a functional coupling of *copia, 412,* and F transposition and some component present in the P-M dysgenic system.

The singed locus is a "hotspot" for P element insertions during P-M hybrid dysgenesis. A number of sn mutations have been induced by this process, which possess different properties in terms of their phenotypic strengths, germline reversion rates, and somatic destabilisation by a certain trans-acting component. Characterisation by Southern and Northern blotting revealed that in each mutant strain the elements were inserted within the 5' transcribed regions of the gene causing a reduction in level of sn poly $(A)^+$ RNA, and also, in one case, the production of two novel mRNA species. It was also determined that the germline and somatic reversion frequencies of the strains were most probably dependent upon the target sites into which the elements were inserted. Repeated attempts to clone two insertions at the sn locus in one of the strains proved unsuccessful, most probably due to the unusual structure exhibited by the elements in this strain.

Postdoctoral research:

"An animal model (*Plasmodium yoelii*) for the development of a vaccine against the against the asexual blood stage of *P.falciparum* based on the precursor to the major merozoite surface antigens".

The gene encoding the 230kDa precursor to the major merozoite surface antigens (PMMSA) of *Plasmodium yoelii* YM has been c cloned, and the complete nucleotide sequence determined. A single open reading frame of 5316 base pairs encodes a polypeptide of calculated molecular mass 197232. The deduced amino

acid sequence contains potential signal peptide and membrane anchor sequences of 19 and 18 residues respectively, and a region of six tandemly repeated tetrapeptides, Gly-Ala-Val-Pro. There are 20 cysteine residues and 11 potential N-glycosylation sites.

Computer analysis was used to compare the amino acid sequence of the *P.yoelii* YM Py230 antigen with the complete sequences of the two Pf195 alleles from the *P.falciparum* Wellcome and MAD20 strains, and also with a published region of the *P.vivax* PMMSA, Pv200. Certain regions of the PMMSA were found to be conserved between the three evolutionary distant malarial species, thus arguing for some important structural and/or functional constraints in these regions. Extensive homologies were found throughout the length of the molecule thus reinforcing the use of Py230 as a rodent model for the PMMSA. The polypeptide was divided into 22 blocks classed as either conserved, semi-conserved or variable, based upon amino acid conservation.

A fragment from the Py230 gene was used to probe an RNA Northern blot of total insertion of sequences from the signal peptide region of a baculovirus capsid protein gp67 before the cloning site. Py230 gene fragments analogous to the regions encoding the Pf195 processing fragments were PCR amplified and cloned into the expression vectors. Py230 regions analogous to the Pf195 42, 38 and 83kDa polypeptides, cloned into a vector possessing just the gp67 signal sequence, were expressed in the baculovirus/insect cells system. The protein products were detected on Western blots using monoclonal antibodies McAb302 and Mab 25.1, which recognise the epitopes within the carboxy- and amino-terminal portions of the Py230 respectively, and rabbit polyclonal Py230 antiserum. The Pf42 and Pf38 analogous polypeptides were found to be situated in cell pellet fractions after centrifugation, whereas the polypeptide analogous to Pf83 was secreted into the culture medium, thus suggesting that sequences immediately downstream of the signal peptide cleavage site may be important for correct secretion. A second vector has thus been constructed possessing the gp67 signal sequence plus an additional 10 downstream amino acids, into which Py230 fragments have been cloned.

The Py230 region analogous to Pf83 was expressed in insect cells and purified from the culture medium using Mab 25.1-Sepharose 4B affinity chromatography. Animal experiments are presently underway in collaboration with C. Long (Hahnemann University, Philadelphia).

Present research:

My present work involves the formulation of novel therapeutic monoclonal antibodies (mAbs), especially in the areas of inflammation and anti-viral therapy, using molecular biological gene manipulation and eukaryotic gene expression techniques. The use of rodent mAbs as human therapeutics has been hampered by the failure of most rodent Ab isotypes to activate human effector mechanisms, and by the immunogenicity of rodent mAbs in humans. This has led to the humanisation of mAbs, that is, the transplantation of the

complementarity-determining regions from the appropriate rodent mAb onto a human immunoglobulin (Ig) framework. I have developed and patented a new technique for mAb humanisation utilising the recombinant polymerase chain reaction. This method has been used to humanise a rat anti-interleukin 2 receptor mAb which may have uses in transplant and autoimmune disease therapy.

Human mAbs would be preferable to rodent mAbs as therapeutic agents. However, although human mAbs have been produced by conventional hybridoma technology and by EBV transformation the general production of such reagents has been dogged by instability of the cell lines and low levels of expression of the mAbs. I have been involved with the development and patenting of a method allowing the rescue of human Igs enabling the immortalisation of functional human mAbs. This technique has been used to rescue an anti-hepatitis A human mAb.

Research is also under progress investigating the use of monkey mAbs as therapeutics.

PUBLICATION LIST
Alan P. Lewis

Lewis, A.P. and Brookfield, J.F.Y. (1987) Movement of *Drosophila melanogaster* transposable elements other than P elements in a P-M hybrid dysgenesis cross. Mol. Gen. Genet. 208:506-510.

Brookfield, J.F.Y. and Lewis, A.P. (1989) Somatic reversion of P transposable element insertion mutations in the *singed* locus of *Drosophila melanogaster* requiring specific P insertions and a *trans*-acting factor. Genet. Res., Camb. 54:101-112.

Lewis, A.P. (1989) The 230000 molecular mass merozoite surface antigen of *Plasmodium yoelii*: cloning and analysis of the 3' half of the gene. J. Cell. Biochem. 13E:142.

Lewis, A.P. (1989) Cloning and analysis of the gene encoding the 230-kilodalton merozoite surface antigen of *Plasmodium yoelii*. Mol. Biochem. Parasitol. 36:271-282.

Lewis, A.P. (1990) Sequence analysis upstream of the gene encoding the precursor to the major merozoite surface antigens of *Plasmodium yoelii*. Mol. Biochem. Parasitol. 39:285-288.

Keen, J., Holder, A., Playfair, J., Lockyer, M. and Lewis, A. (1990) Identification of the gene for a *Plasmodium yoelii* rhoptry protein. Multiple copies in the parasite genome. Mol. Biochem. Parasitol. 42:241-246.

Lewis, A.P. and Crowe, J.S. (1991) Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate humanised monoclonal antibodies. Gene 101:297-302.

Lewis, A.P., Parry, N., Peakman, T.C. and Crowe, J.S. (1992) Rescue and expression of human immunoglobulin genes to generate functional human monoclonal antibodies. Hum. Antibod. Hybridomas. 3:146-152.

Gewert, D., Salom, C., Barber, K., Macbride, S., Cooper, H., Lewis, A., Wood, J. and Crowe, S. (1993) Analysis of interferon-α2 sequences in human genomic DNA. J. Interferon Res. 13:227-231.

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Sims, M.J., Hassal, D.G., Brett, S., Rowan, W., Lockyer, M.J., Angel, A., Lewis, A.P., Hale, G., Waldmann, H. and Crowe, J.S. (1993) A humanized CD18 antibody can block function without cell destruction. J. Immunol. 151:2296-2308.

Lewis, A.P., Lemon, S.M., Barber, K.A., Murphy, P., Parry, N.R., Peakman, T.C., Sims, M.J., Worden, J. and Crowe, J.S. (1993) Rescue, expression, and analysis of a neutralizing human anti-hepatitis A virus monoclonal antibody. J. Immunol. 151:2829-2838.

Lewis, A.P., Barber, K.A., Cooper, H.J., Sims, M.J., Worden, J. and Crowe, J.S. (1993) Cloning and sequence analysis of κ and γ cynomolgus monkey immunoglobulin cDNAs. Developmental Comp. Immunol. 17:549-560.

Crowe, J.S., Gewert, D.R, Barber, K.A., Lewis, A.P., Sims, M.J., Davies, S.L., Salom, C.L., Wood, J., Thomas, H.C., Thursz, M. and Lok, A.S. (1994) Interferon (IFN)-α2 genotype analysis of Chinese chronic hepatitis B patients undergoing recombinant IFN-α2a therapy. J. Infect. Dis. 169:875-878.

Lewis, A.P., Sims, M.J., Gewert, D.R., Peakman, T.C., Spence, H. and Crowe, J.S. (1994) *Taq* polymerase extension of internal primers blocks polymerase chain reactions allowing differential amplification of molecules with identical 5' and 3' ends. Nucleic Acids Res. 22:2859-2861.